510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number: k040198

B. Analyte: benzylecgonine (cocaine metabolite)

C. Type of Test: enzyme immunoassay (microplate)

D. Applicant: LabOne Inc.

E. Proprietary and Established Names: LabOne Cocaine Metabolite Micro-Plate EIA

F. Regulatory Information:

1. Regulation section:
   21CFR862.3250, Cocaine and Cocaine Metabolite Test System
2. Classification:
   Class II
3. Product Code:
   91 DIO
4. Panel:
   Toxicology

G. Intended Use:

1. Indication(s) for use:
   The LabOne MicroPlate Cocaine Enzyme Immunoassay (EIA) is intended for the qualitative determination of cocaine and cocaine metabolites in oral fluid collected with the OraSure® Oral Fluid Specimen Collection Device. It is a screen test with a cutoff set at 10 ng/ml of benzylecgonine per ml of oral fluid. (See cross-reactivity section for information on cross-reactivity with cocaine). The test is intended for laboratory use only. For in vitro diagnostic use.

   The LabOne Cocaine Metabolite Microplate EIA Calibrators are intended for medical purposes for use with the LabOne Cocaine Metabolite Microplate EIA to establish points of reference that are used in determination of values in the measurement of cocaine in saliva.

   The LabOne Cocaine Metabolite Microplate EIA Controls are intended for use as an assayed quality control matrix to monitor the precision and accuracy of the laboratory testing procedures for cocaine metabolite.

2. Special condition for use statement(s):
   The assay provides only a preliminary analytical test result. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly in evaluating a preliminary positive result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/Mass spectrometry is the recommended confirmatory method.

   For prescription use only

3. Special instrument Requirements:
   Microplate reader
   OraSure® Oral Fluid Specimen Collection device.

H. Device Description:
Reagents and components included in the device are microplates, enzyme conjugate concentrate, diluent, substrate solutions, stop solution, wash concentrate, calibrators and controls.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   STC Cocaine Metabolite Microplate

2. Predicate K number(s):
   k973651

3. Comparison with predicate:
   The devices are similar in terms of intended use and test principle. The anti-benzylecgonine antibodies differ. The predicate device uses a monoclonal antibody; this device uses a polyclonal antibody. Cross-reactivity with cocaine is higher with this device (77%) than with the predicate device (12%).

J. Standard/Guidance Document Referenced (if applicable):"Statistical Guidance on Reporting Results From Studies Evaluating Diagnostic Tests; draft guidance for industry and FDA reviewers", CDRH, FDA, March 12, 2003; "Premarket Submission and Labeling Recommendations for Drugs of Abuse Screening Tests", draft guidance by CDRH, FDA, December 2, 2003; "In Vitro Diagnostic Device: Guidance for the Preparation of 510(k) submissions", HHS publication FDA 97-4224.

K. Test Principle:
The LabOne Cocaine Metabolite Micro-Plate EIA is a competitive micro-plate immunoassay. Cocaine and cocaine metabolite (benzylecgonine) in the oral fluid compete with cocaine metabolite in the enzyme conjugate for binding sites on the antibody that is coated on the solid surface of the micro-well. The absorbance (read at 450 nm) produced by the assay reagents is inversely proportional to the amount of cocaine metabolite in the specimen, calibrator or control. A negative

calibrator, cutoff calibrator and controls are run with the assay. The manufacturer advises to run all samples in duplicate.

L. Performance Characteristics (if/when applicable):

All performance was established on the Titertek Multiskan MCC/340 plate reader at the manufacturer's site. All samples were processed as per instructions for the OraSure® Oral Fluid Specimen Collection device.

1. Analytical performance:

a. Precision/Reproducibility:

A negative oral fluid pool was prepared by pooling more than 50 negative samples. Samples were spiked to levels of 0, 5, 10 and 20 ng/ml. Testing was over 20 days, with six replicates per day for each sample. Three product lots were included in the testing. Three separate lots were used and 3 technicians performed the precision testing (Each of the 3 technicians performed the test with a different lot for 6-7 days.)

Intra-assay coefficients of variation (cv's) were calculated as the average of the 6 daily intra-assay %cv's for each sample. Inter-assay cv's were calculated as averages of the 120 results obtained for each level tested.

Intra-Assay Precision Results:

	0 ng/ml BE	5 ng/ml BE	10 ng/ml BE	20 ng/ml BE
Mean OD	1.714	1.186	1.025	0.919
SD (OD units)	0.043	0.038	0.035	0.027
N	120	120	120	120
Mean %CV	2.50	3.28	3.45	2.99

Inter-Assay Precision Results:

	0 ng/ml BE	5 ng/ml BE	10 ng/ml BE	20 ng/ml BE
Average Normalized OD	1.000	0.686	0.594	0.503
SD (OD units)	0.027	0.036	0.034	0.034
N	120	120	120	120
%CV	2.67	5.21	5.79	6.76

An additional evaluation of near-cutoff samples was performed with spiked pools of negative oral fluid specimens. Specimen pools were spiked to final concentrations of near 0 (<1), 5+/-0.75, 7.5 (6.4-8.6), 12.5 (10.6-14.4) and 15 (12.8-17.3) ng/ml. Mean values of 20 to 30 replicates were determined. These are tabulated below:

Evaluation of Near-Cutoff Study Samples:

	0 ng/ml BE	5 ng/ml BE	7.5 ng/ml BE	12.5 ng/ml BE	15 ng/ml BE
Cutoff OD	1.449	1.449	1.402	1.402	1.402
Sample mean OD	2.554	1.720	1.573	1.348	1.209
SD (OD units)	0.0665	0.0415	0.0660	0.0442	0.0548
N	30	30	30	20	20
%CV	2.60	2.41	4.19	3.28	4.54

b. Linearity/assay reportable range: Not applicable. This is a qualitative assay.

c. Traceability (controls, calibrators, or method):
Calibrators and controls contain benzylecgonine in citric buffered saline with detergent and stabilizing protein. Stocks for calibrators and controls are prepared gravimetrically from purified commercially available benzylecgonine. The stock is assigned based on replicate GCMS analyses. Calibrators and controls are prepared by diluting the stock into the matrix. Values of cutoff calibrators are confirmed by GCMS. Acceptance criteria are values within +/- 15% of 10 ng/ml.

Real-time stability is determined for opened and closed calibrators stored at 2-8 degrees C for 3 months. Acceptance criteria are values within +/- 10% of fresh calibrator.

d. Detection limit:
The detection limit is defined as a sample with an optical density that is 2 standard deviations above the mean of a zero sample. The limit of detection determined for this assay is well below the validated cutoff value of 10 ng/ml.

e. Analytical specificity:
To evaluate potential interference from common drugs, endogenous compounds and several consumable substances, negative (2.5

ng/ml) and positive (15 ng/ml) oral fluid specimen pools were spiked with 10 ug/ml of potential interferent. No unusual interference from the drugs tested was observed at these concentrations. The full list of compounds tested is included in the product package insert.

The effect of endogenous compounds including albumin (0-5 ug/ml) bilirubin (up to 50 mg/L), ascorbic acid (up to 10,000 mg/L) and hemoglobin (up to 1000 mg/L) was evaluated. Bilirubin, hemoglobin and albumin had no effect on positive or negative test results at these concentrations. Ascorbic acid at concentrations above 5000 mg/L caused false positive results.

A variety of consumable substances (including 3.3% w/v sucrose , 33% v/v cranberry juice, 3.3% w/v baking soda, 33% v/v orange juice, cola, cough syrup, antiseptic mouth wash, 0.05% w/v toothpaste, 40ug/ml dental adhesive A and 40 ng/ml dental adhesive B) were evaluated for interference. Orange juice caused false positive results. The other compounds tested did not affect results at these concentrations.

The effect of pH on test results was similarly evaluated. No effect was seen within the pH range 5-10 on positive or negative samples. (Samples outside this range produced false results.)

The following structurally related compounds cross-reacted at the levels listed below. These values were determined by serial dilutions of (duplicate) samples to obtain concentrations at which gives signals were equivalent to 10 ng/ml benzylecgonine.

compound	% cross-reactivity
cocaethylene	73%
cocaine	77%
ecgonine methyl ester hydrochloride	0.1%
ecgonine hydrochloride	1%

The effect of matrix condensation was evaluated by condensing and diluting sample matrices up to 50% and then spiking to benzylecgonine concentrations of 0, 2.5 and 15 ng/ml. No effect of matrix condensation or dilution was observed on positive or negative samples.

f. Assay cut-off:

See the evaluation of near cutoff samples within the precision section above.

2. Comparison studies:
    a. *Method comparison with predicate device*
    One hundred and eight specimens were collected from volunteers from a population suspected to have a high prevalence of cocaine use. To supplement the near-cutoff samples, another 18 samples were prepared by diluting individual specimens. Collection and processing was performed by professionals at LabOne. All samples were collected with Orasure Oral Fluid Collection Device. Duplicates of each sample were evaluated and compared to results of GC/MS/MS analysis. The sum of benzylecgonine and cocaine concentrations were determined in this analysis. Results are tabulated below:

GC/MS BE+cocaine (ug/ml)	Range	Negative <5.0 ng/ml	Near cutoff negative 5.0-9.9 ng/ml	Near cutoff positive 10-15 ng/ml	Positive >15.0 ng/ml
LabOne EIA	positive	0	3	5	17
	negative	88	9	3	1

Positive agreement: 84.6% (95% confidence interval : 65%-96%)
Negative agreement 97.3% (95% confidence interval : 92%-99%)

    b. *Matrix comparison:*
    The device is intended for use only with oral fluid only.

3. Clinical studies:
    a. *Clinical sensitivity:* N/A. (Not typically reviewed for this type of test.)

    b. *Clinical specificity:* N/A. (Not typically reviewed for this type of test.)

4. Clinical cut-off: The cutoff of 10 ng/ml is the same as that of the predicate device.

5. Expected values/Reference range: N/A

M. Conclusion:

I recommend that the The LabOne MicroPlate Cocaine Enzyme Immunoassay (EIA) is substantially equivalent to the predicate device.